Stavrianopolous, et al

Serial No.: 10/764,389

Filed: January 23, 2004

Page 3 [Amendment in Response to Notice to Comply With Sequence

Listing Rules – June 8, 2004]

In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 31, lines 10-11, and replace it with the

following paragraph:

FIGURE 4 depicts variations of placement of primers (SEQ ID NO: 5) in a

double stranded nucleic acid.

Please delete the paragraph on page 31, lines 22-24, and replace it with the

following paragraph:

FIGURE 12 shows the sequence of an HIV antisense amplicon (SEQ ID NO:

8) and sequences of two primers (SEQ ID NOS 6 & 9) and one probe (SEQ ID NO:

7) used in the examples below to illustrate the novel use of energy transfer in the

present invention.

Please delete the paragraph on page 31, lines 25-26, and replace it with the

following paragraph:

FIGURE 13 shows the use of a CNAC (SEQ ID NO: 4) to eliminate a portion

of a poly A tail followed by incorporation of an oligo C primer binding sequence

(SEQ ID NOS 10-12).

Please delete the paragraph on page 100, lines 20-29, thru page 101, lines 1-7,

and replace it with the following paragraph:

EXAMPLE 10 Energy transfer between an intercalator and an incorporated

dye with primers that comprise quencher moieties

Enz-61(D8)

Stavrianopolous, et al

Serial No.: 10/764,389

Filed: January 23, 2004

Page 4 [Amendment in Response to Notice to Comply With Sequence

Listing Rules - June 8, 2004]

This example is carried out as describe in Example 9 except that the primers are labeled with quenchers as follows:

5' CAU*GATCCGGAU*GGGAGGTG 3' (SEQ ID NO: 1) and

5' GCACAU*CCGGAU*AGU*AGA 3' (SEQ ID NO: 2)

where U* are uridine moieties modifed with a non-fluorescent 3-amino xanthene as described by Singer and Haugland in U.S. Patent No. 6,323,337 that absorb at about 530 nm. PCR is carried out with these primers in the presence of a labeled dUTP from Example 7 and SYBR Green as described above. Fluorescence from the intercalated SYBR Green can be absorbed either by Compound XVIII or by the quencher. If Primer-dimers are formed, these comprise only primers and their complements. As such energy transfer should most efficiently take place with the quenchers and thereby reduce spurious signal generation from primer-dimer synthesis. On the other hand, amplicons derived from amplfication of target sequences have segments where only compound XVIII is in sufficient proximity to the SYBR for energy transfer to take place and target dependent signals are generated as synthesis proceeds.

Please delete the paragraph on page 101, lines 8-20, and replace it with the following paragraph:

EXAMPLE 11 Energy transfer between a probe and an incorporated nucleotide

PCR can be carried out with the same primers used in Example 8. In this reaction mixture, potential donors are supplied in the form of dUTP labeled with Compound XVIII form Example 7. The reaction mixture also contains a DNA probe labeled with Texas Red moieties that can act as energy acceptors. The probe has the sequence

Stavrianopolous, et al

Serial No.: 10/764,389

Filed: January 23, 2004

Page 5 [Amendment in Response to Notice to Comply With Sequence

Listing Rules - June 8, 2004]

5' UFAATGGUFGAGTATCCCUFGCCTAACTCUF 3' (SEQ ID NO: 3)

where U^F indicates a Uridine labeled with Texas Red. The position of this probe in the amplicon is shown in Figure 12. The probe is also blocked at the 3' end such that it is incapable of being extended. As amplification is carried out, hybridization of the probe to labeled amplicon strands allow energy transfer to take place between Compound XVIII and Texas Red that should increase as more amplicon strands are generated.

Please delete the paragraph on page 101, lines 21-29, thru page 102, lines 1-8, and replace it with the following paragraph:

EXAMPLE 12 Endonuclease digestion and strand extension using a

homopolymeric target as a substrate

The steps in this example are shown in Figure 13. A CNAC with three segments can be synthesized that has the sequence:

5'-UUUUUUUUUTTTTQQQQQQQQ-3' (SEQ ID NO: 4)

where U is a uridine ribonucleotide, T is a thymidine deoxyribonucleotide and Q is an inosine ribonucleotide and the 3' end has been modifed to prevent extension. In this example, the ribonuicleotides are 2'-O-methyl as described by Shibahara et al., (1987) Nucl. Acids Res.15; 4403-4415 and Baranov et al., (1997) Nucl. Acids Res. 25; 2266-2273 (both of which are incorporated by reference). The CNAC can be hybridized to a library of poly A mRNA (step A) forming:

a first complex with the oligo-uridine first segment bound to a portion of the poly A tail,

a second complex with the oligo-thymidine second segment bound to a second portion of the poly A tail; and

a third complex with the oligo-inosine third segment bound to a third portion of the poly A tails.